REMARKS

I. Nationalization

This application represents the U.S. national stage of International Patent Application PCT/NZ00/00179, filed September 14, 2000, which claims priority to New Zealand priority Application 337792, filed September 14, 1999.

As the text of the International Application was transmitted by the International Bureau, an additional copy is not required to satisfy 35 U.S.C. § 371(c)(2). Nonetheless, for the Examiner's convenience, a copy of international application PCT/AU99/00813 is enclosed in the form of the published PCT Application WO 00/18790.

Should formal amendments to the specification be necessary to conform to U.S. practice, Applicants seek to introduce such amendments into the present specification by, *e.g.*, deleting the PCT cover page, providing the Abstract as a separate page, and deleting the PCT header.

Priority is also properly claimed by an amendment at page 1.

Amendments were made to the application during PCT examination, both to the specification and claims. However, none of the amendments to the specification or claims during submitted PCT examination should be entered upon entry into the U.S. national stage. Therefore, the original, unamended PCT application forms the basis for the amendments introduced herein.

II. National Stage Claims

After according a U.S. filing date, and **before** calculating the filing fee, entry of the foregoing claim amendments is respectfully requested.

The claims do not represent the claims at the IPER stage, but the text of the claims when the PCT application was filed. The original PCT claims therefore form the basis for the present

claim amendments, which are of a procedural nature only, revising the claims to better accord with U.S. practice.

The revised claims are fully supported by the specification and claims of the international application and do not in any way constitute new matter.

III. Status of the Claims

The PCT application was filed with claims 1-55, which were pending prior to the present amendment. PCT examination indicated each of claims 1-56 to have unity of invention, which should be noted upon entry into the into the U.S. national stage.

Presently, claims 47 and 48 have been canceled without prejudice and disclaimer, as not being in accordance with U.S. practice. Claims 3-6, 9, 10, 12, 14, 16-18, 20-24, 26, 27, 30, 32-34, 36-40, 43-45, 49 and 51-55 have been amended without prejudice and disclaimer, to better accord with U.S. practice, *e.g.*, to remove multiple dependencies and to make clerical changes. No claims have been added. Claims 1-46 and 49-55 are therefore in the case.

IV. Support for the Claims

Aside from removing the multiple dependencies throughout, and introducing minor changes, current claims 1-46 and 49-55 represent those of the PCT application as filed, essentially in unamended form.

Most of the changes to the revised claims simply remove the multiple dependencies, and such changes are clearly supported by each claim itself.

In addition, claim 17 and claim 34 have been revised to delete the phrases "using methods well known in the art" and "by methods known in the art", respectively, as being redundant in U.S. claim practice.

Claims 20-23 have been revised to add the term "non-human", so that the claims recite "non-human animal embryo".

It will therefore be understood that no new matter is encompassed by any of the present amendments.

V. Compliance with 37 C.F.R. § 1.121

Copies of the pending claims are attached hereto as **Exhibit A** and **Exhibit B**. In accordance with 37 C.F.R. § 1.121, the claims have been labeled as "(Amended"), where appropriate. **Exhibit A** provides a clean copy of the pending claims, whereas **Exhibit B** shows the changes with brackets and underlining.

The proper claim for priority has been timely introduced into the specification by amendment of the opening paragraph at page 1. An Abstract is also introduced into the specification by amendment as a separate page.

The amendments to the specification have been made as "replacement paragraphs" in accordance with 37 C.F.R. § 1.121. This is proper as the amendments include the reference, replacement paragraph in clean form and another version of the replacement paragraph separate from the amendment marked up to show all changes (**Exhibit C**).

VI. Fees and Formalities

The national filing fee and claim fees are included herewith. The fees have been calculated **after** the present changes to remove the multiple dependencies throughout the claims. Any omitted fees should be deducted from Williams, Morgan & Amerson Deposit Account No. 50-0786/4070.000300.

Applicants are believed to be required to pay large entity fees, but reserve the right to request a refund should this prove to be in error.

Should the Office have any questions or comments, a telephone call to the undersigned Applicant's representative is earnestly solicited.

Respectfully submitted,

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Date: March 14, 2002

EXHIBIT A
NATIONAL STAGE CLAIMS

1. A method of nuclear transfer, comprising selecting and segregating G1 cells from a proliferating or non-proliferating population of donor cells and transferring a nucleus from such a segregated G1 cell into an enucleated recipient cell.

2. A method as claimed in claim 1, wherein the donor cell population is at one or more known or unknown stages of the cell cycle.

3. (Amended) A method as claimed in claim 1, wherein said donor cell population is non-proliferating and has been synchronised at any point in the G1 stage of the cell cycle.

4. (Amended) A method as claimed in claim 1, wherein said G1 cell is segregated at an early G1 phase.

5. (Amended) A method as claimed in claim 1, wherein the donor cell population is non-proliferating and comprises senescent cells.

6. (Amended) A method as claimed in claim 1, wherein said donor cell population is derived from either embryo, fetal, juvenile or adult cells isolated from an animal *in vivo* or from a cell culture *in vitro*.

7. A method as claimed in claim 6, wherein said donor cell population comprises any diploid karyotypically normal cell capable of being stimulated to enter the cell cycle and proliferate.

8. A method as claimed in claim 7, wherein said donor cell population is of an undifferentiated cellular state or are at any degree of differentiation or quiescence or senescence.

9. (Amended) A method as claimed in claim 1, wherein the donor cells are adult or fetal fibroblasts or follicular cells.

10. (Amended) A method as claimed in claim 1, wherein said donor cells comprise modified cells.

11. A method as claimed in claim 10 wherein said donor cells comprise transgenic cells.

12. (Amended) A method as claimed in claim 1, wherein the recipient cell comprises an enucleated oocyte.

13. A method as claimed in claim 12, wherein the enucleated oocyte is obtained from a species corresponding in origin to the donor nuclei.

14. (Amended) A method as claimed in claim 1, wherein the recipient cell comprises an enucleated stem cell or a clump of enucleated stem cells fused together.

15. A method as claimed in claim 14, wherein the stem cells are embryonic stem cells isolated from a growing embryo or form an established cell line in culture.

16. (Amended) A method of producing cloned animal embryos which comprises transferring a segregated donor nucleus in the G1 stage of the cell cycle into an enucleated recipient cell.

17. (Amended) A method as claimed in claim 16, wherein the donor nuclei are genetically altered to produce cloned embryos having desirable genetic traits.

18. (Amended) A method as claimed in claim 16, when used to produce an animal species of cloned embryo selected from the group comprising birds, amphibia, fish and mammals.

19. A method as claimed in claim 18, wherein said cloned animal embryo is a mammal, selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

20. (Amended) A reconstituted non-human animal embryo prepared by the method claimed in claim 16.

21. (Amended) A reconstituted non-human animal embryo as claimed in claim 20, comprising a transgenic embryo.

22. (Amended) A reconstituted non-human animal embryo as claimed in claim 20 re-cloned to further increase embryo numbers or which undergoes serial nuclear transfer to aid nuclear reprogramming and/or development.

23. (Amended) A reconstituted non-human animal embryo as claimed in claim 20, comprising a species of mammal selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

24. (Amended) A method of cloning a non-human animal comprising the steps: (1) producing a cloned non-human animal embryo according to the method of claim 16; (2) allowing a non-human animal to develop to term from the embryo; and (3) optionally breeding from the non-human animal so formed either by conventional methods or by further cloning.

25. A method as claimed in claim 24, wherein said cloned non-human animal is a non-human mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

26. (Amended) A method as claimed in claim 24, wherein said cloned non-human animal is a transgenic non-human animal having a desirable genetic trait.

27. (Amended) A method as claimed in claim 26, wherein said transgenic non-human animal is a transgenic bovine or ovine.

28. A cloned non-human animal prepared by the method of claim 24.

29. A cloned non-human animal as claimed in claim 28 comprising a mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

30. (Amended) A cloned non-human animal as claimed in claim 28, comprising a transgenic non-human animal having a desirable genetic trait.

31. A cloned non-human animal as claimed in claim 30 comprising a transgenic bovine or ovine.

32. (Amended) A cloned non-human transgenic animal as claimed in claim 30, wherein the desirable genetic trait is selected from the insertion, deletion, or modification of a gene or genes enabling the production of pharmaceutical proteins in milk, blood or urine; production of nutraceutical products in milk or meat; production of beneficial agricultural traits to improve the quality of milk, meat and fibre production; improve resistance to pests and diseases; production of industrial proteins in milk; xenotransplantation; and for the generation of transgenic animals as models for human disease.

33. (Amended) Offspring and descendants of the cloned non-human animal as claimed in claim 28.

34. (Amended) A method of producing an embryonic cell line comprising the steps a) selecting and segregating G1 cells from a proliferating population of donor cells or from a synchronised population of G1 cells or from a population of senescent cells, and transforming a nucleus from such a segregated cell into an enucleated recipient cell; b) growing to blastocyst stage; c) recovering embryonic cells; and d) establishing an immortalised cell line *in vitro*.

35. A method as claimed in claim 34, wherein said embryonic cells are embryonic stem cells.

36. (Amended) A method as claimed in claim 34, wherein said donor cells are human cells.

37. (Amended) A method as claimed in claim 34, wherein both donor and recipient cells are human cells.

38. (Amended) A method as claimed in claim 34, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

39. (Amended) An embryonic cell line produced by the method of claim 34.

40. (Amended) A human embryonic stem cell line produced by the method of claim 35, useful in therapeutic applications.

41. A method of producing embryonic stem cells comprising the steps of a) selecting and segregating G1 cells from a proliferating population of donor cells or from synchronised population of G1 cells or from a population of senescent cells and transferring a nucleus from

such a segregated cell into an enucleated recipient cell; b) growing to blastocyst stage; and c) recovering embryonic stem cells.

42. A method as claimed in claim 41, wherein said donor cells are human cells.

43. (Amended) A method as claimed in claim 41, wherein both donor and recipient cells are human cells.

44. (Amended) A method as claimed in claim 41, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

45. (Amended) Embryonic stem cells produced by the method of claim 41.

46. Embryonic stem cells as claimed in claim 45, comprising human embryonic stem cells.

49. (Amended) A method of therapeutic cloning, wherein embryonic stem cells are produced according to claim 35 from a donor cell derived from a subject, and cultured to produce specialised cells or tissue for transplantation in said subject or in another subject in need of such treatment.

50. A method as claimed in claim 49, wherein said embryonic stem cells comprise one or more transgenes to confer a desirable genetic trait in the resulting differentiated cells used for transplantation.

51. (Amended) A method of treating a disease, disorder or injury which may be treated by transplantation of specialised cells or tissue, comprising administering to a patient in need thereof a therapeutically effective amount of specialised cells or tissue produced according to the method of claim 49.

52. (Amended) A method as claimed in claim 49, wherein said disease, disorder or injury is selected from various neurological disorders (*eg* Parkinson's disease), diabetes, heart disease, muscular dystrophy, various hereditary diseases, specific cancers (*eg* leukemia), spinal cord injury, burns and other afflictions.

53. (Amended) A method of drug discovery or toxicology testing of drugs using *in vitro* differentiated human embryonic stem cells produced by the methods of claim 41.

54. (Amended) A method of xenotransplantation, wherein cells, tissues and organs are isolated from the non-human cloned animal of claim 28, and used for transplantation in a human patient in need thereof.

55. (Amended) A method of gene therapy, wherein cells, tissues and organs comprise a transgene and are isolated for the non-human cloned animal of claim 30.

EXHIBIT B
NATIONAL STAGE CLAIMS

1. A method of nuclear transfer, comprising selecting and segregating G1 cells from a proliferating or non-proliferating population of donor cells and transferring a nucleus from such a segregated G1 cell into an enucleated recipient cell.

2. A method as claimed in claim 1, wherein the donor cell population is at one or more known or unknown stages of the cell cycle.

3. (Amended) A method as claimed in claim 1 [or 2], wherein said donor cell population is non-proliferating and has been synchronised at any point in the G1 stage of the cell cycle.

4. (Amended) A method as claimed in [any one of claims 1 to 3] claim 1, wherein said G1 cell is segregated at an early G1 phase.

5. (Amended) A method as claimed in [any one of claims 1 to 3] claim 1, wherein the donor cell population is non-proliferating and comprises senescent cells.

6. (Amended) A method as claimed in [any one of claims 1 to 5] claim 1, wherein said donor cell population is derived from either embryo, fetal, juvenile or adult cells isolated from an animal *in vivo* or from a cell culture *in vitro*.

7. A method as claimed in claim 6, wherein said donor cell population comprises any diploid karyotypically normal cell capable of being stimulated to enter the cell cycle and proliferate.

8. A method as claimed in claim 7, wherein said donor cell population is of an undifferentiated cellular state or are at any degree of differentiation or quiescence or senescence.

9. (Amended) A method as claimed in [any preceding] claim 1, wherein the donor cells are adult or fetal fibroblasts or follicular cells.

10. (Amended) A method as claimed in [any preceding] claim 1, wherein said donor cells comprise modified cells.

11. A method as claimed in claim 10 wherein said donor cells comprise transgenic cells.

12. (Amended) A method as claimed in [any preceding] claim 1, wherein the recipient cell comprises an enucleated oocyte.

13. A method as claimed in claim 12, wherein the enucleated oocyte is obtained from a species corresponding in origin to the donor nuclei.

14. (Amended) A method as claimed in [any one of claims 1 to 11] claim 1, wherein the recipient cell comprises an enucleated stem cell or a clump of enucleated stem cells fused together.

15. A method as claimed in claim 14, wherein the stem cells are embryonic stem cells isolated from a growing embryo or form an established cell line in culture.

16. (Amended) A method of producing cloned animal embryos which comprises transferring a segregated donor nucleus in the G1 stage of the cell cycle into an enucleated recipient cell.

17. (Amended) A method as claimed in claim 16, wherein the donor nuclei are genetically altered [using methods well known in the art] to produce cloned embryos having desirable genetic traits.

18. (Amended) A method as claimed in claim 16 [or 17], when used to produce an animal species of cloned embryo selected from the group comprising birds, amphibia, fish and mammals.

19. A method as claimed in claim 18, wherein said cloned animal embryo is a mammal, selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

20. (Amended) A reconstituted non-human animal embryo prepared by the method claimed in claim 16.

21. (Amended) A reconstituted non-human animal embryo as claimed in claim [17] 20, comprising a transgenic embryo.

22. (Amended) A reconstituted non-human animal embryo as claimed in claim 20 [or 21] re-cloned to further increase embryo numbers or which undergoes serial nuclear transfer to aid nuclear reprogramming and/or development.

23. (Amended) A reconstituted non-human animal embryo as claimed in [any one of claims 20 to 22] claim 20, comprising a species of mammal selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

24. (Amended) A method of cloning a non-human animal comprising the steps: (1) producing a cloned non-human animal embryo according to the method of [any one of claim 16 or 17] claim 16; (2) allowing a non-human animal to develop to term from the embryo [by known methods]; and (3) optionally breeding from the non-human animal so formed either by conventional methods or by further cloning.

25. A method as claimed in claim 24, wherein said cloned non-human animal is a non-human mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

26. (Amended) A method as claimed in claim 24 [or 25], wherein said cloned non-human animal is a transgenic non-human animal having a desirable genetic trait.

27. (Amended) A method as claimed in claim 26, wherein said transgenic non-human animal is a transgenic bovine or ovine.

28. A cloned non-human animal prepared by the method of claim 24.

29. A cloned non-human animal as claimed in claim 28 comprising a mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

30. (Amended) A cloned non-human animal as claimed in claim 28 [or 29], comprising a transgenic non-human animal having a desirable genetic trait.

31. A cloned non-human animal as claimed in claim 30 comprising a transgenic bovine or ovine.

32. (Amended) A cloned non-human transgenic animal as claimed in claim 30 [or 31], wherein the desirable genetic trait is selected from the insertion, deletion, or modification of a gene or genes enabling the production of pharmaceutical proteins in milk, blood or urine; production of nutraceutical products in milk or meat; production of beneficial agricultural traits to improve the quality of milk, meat and fibre production; improve resistance to pests and diseases; production of industrial proteins in milk; xenotransplantation; and for the generation of transgenic animals as models for human disease.

33. (Amended) Offspring and descendants of the cloned non-human animal as claimed in [any one of claims 28 to 32] claim 28.

34. (Amended) A method of producing an embryonic cell line comprising the steps a) selecting and segregating G1 cells from a proliferating population of donor cells or from a synchronised population of G1 cells or from a population of senescent cells, and transforming a nucleus from such a segregated cell into an enucleated recipient cell; b) growing to blastocyst stage; c) recovering embryonic cells; and d) establishing an immortalised cell line *in vitro* [by methods known in the art].

35. A method as claimed in claim 34, wherein said embryonic cells are embryonic stem cells.

36. (Amended) A method as claimed in claim 34 [or 35], wherein said donor cells are human cells.

37. (Amended) A method as claimed in [any one of claims 34 to 36] claim 34, wherein both donor and recipient cells are human cells.

38. (Amended) A method as claimed in [any one of claims 34 to 37] claim 34, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

39. (Amended) An embryonic cell line produced by the method of [any one of claims 34 to 36] claim 34.

40. (Amended) A human embryonic stem cell line produced by the method of [claim 36, when dependent upon] claim 35, useful in therapeutic applications.

41. A method of producing embryonic stem cells comprising the steps of a) selecting and segregating G1 cells from a proliferating population of donor cells or from synchronised population of G1 cells or from a population of senescent cells and transferring a nucleus from such a segregated cell into an enucleated recipient cell; b) growing to blastocyst stage; and c) recovering embryonic stem cells.

42. A method as claimed in claim 41, wherein said donor cells are human cells.

43. (Amended) A method as claimed in [any one of claims 41 to 42] claim 41, wherein both donor and recipient cells are human cells.

44. (Amended) A method as claimed in [any one of claims 41 to 43] claim 41, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

45. (Amended) Embryonic stem cells produced by the method of [any one of claims 41 to 43] claim 41.

46. Embryonic stem cells as claimed in claim 45, comprising human embryonic stem cells.

Please cancel claims 47 and 48

. A use of the embryonic cells of any one of claims 39, 40 and 45, wherein specialised types of cell or tissue selected from the group comprising nerve cells, muscle cells, heart cells, liver cells, lung cells, kidney cells or any other type of cell of interest are cultured using methods well known in the art.

. A use as claimed in claim 47, wherein said embryonic cells are human embryonic stem cells as claimed in claim 40 or 46.

49. (Amended) A method of therapeutic cloning, wherein embryonic stem cells are produced according to [any one of claims 35 and 41 to 43] claim 35 from a donor cell derived from a subject, and cultured to produce specialised cells or tissue for transplantation in said subject or in another subject in need of such treatment.

50. A method as claimed in claim 49, wherein said embryonic stem cells comprise one or more transgenes to confer a desirable genetic trait in the resulting differentiated cells used for transplantation.

51. (Amended) A method of treating a disease, disorder or injury which may be treated by transplantation of specialised cells or tissue, comprising administering to a patient in need thereof a therapeutically effective amount of specialised cells or tissue produced according to the method of claim 49 [or 50].

52. (Amended) A method as claimed in claim 49 [or 50], wherein said disease, disorder or injury is selected from various neurological disorders (*eg* Parkinson's disease), diabetes, heart disease, muscular dystrophy, various hereditary diseases, specific cancers (*eg* leukemia), spinal cord injury, burns and other afflictions.

53. (Amended) A method of drug discovery or toxicology testing of drugs using *in vitro* differentiated human embryonic stem cells produced by the methods of claim [47] 41.

54. (Amended) A method of xenotransplantation, wherein cells, tissues and organs are isolated from the non-human cloned animal of [any one of claims 28 to 32] claim 28, and used for transplantation in a human patient in need thereof.

55. (Amended) A method of gene therapy, wherein cells, tissues and organs comprise a transgene and are isolated for the non-human cloned animal of claim 30 [or 31].

EXHIBIT C

REPLACEMENT PARAGRAPHS

At page 1, after the title, the additions are as shown:

The present application is a nationalization of International Patent Application PCT/NZ00/00179, filed September 14, 2000, which claims priority to New Zealand priority Application 337792, filed September 14, 1999.

Field of the Invention

The present invention concerns a novel method of nuclear transfer, specifically, although by no means exclusively for use in cloning technologies for the production of mammalian embryos, fetuses and offspring, including genetically engineered or transgenic mammalian embryos, fetuses and offspring.

At page 1, after the title, the final text is as follows:

The present application is a nationalization of International Patent Application PCT/NZ00/00179, filed September 14, 2000, which claims priority to New Zealand priority Application 337792, filed September 14, 1999.

Field of the Invention

The present invention concerns a novel method of nuclear transfer, specifically, although by no means exclusively for use in cloning technologies for the production of mammalian embryos, fetuses and offspring, including genetically engineered or transgenic mammalian embryos, fetuses and offspring.

After page 46, please start another page (47), and insert the following heading and the following text of the Abstract, as taken from the cover page of the PCT application:

ABSTRACT

The present invention provides a method of nuclear transfer by selecting and segregating G1 cells from a donor cell population. This method is advantageous over the prior art as it provides certainty as to the stage of the cell cycle which the donor nuclei are in and allows for the production of cloned transgenic or non-transgenic embryonic cells, reconstituted embryos and whole animals for agricultural, pharmaceutical, nutraceutical and biomedical applications.